AMENDMENTS TO THE SPECIFICATION

[0027] Fig. 2 is a chart showing the amino acid composition of an MMP-9-~~inhibiging~~ inhibiting activity fraction obtained by gel filtration HPLC in Example 1;

[0030] Fig. 5 is a chart of gel filtration (~~Superdex~~ SUPERDEX® 75; Spherical composite of cross-linked agarose and dextran with a resolution range of 3000-70000 Daltons for globular proteins);

[0031] Fig. 6 is an electrophoretogram showing the results of examination of MMP-9-inhibiting activity for the respective gel filtration (~~Superdex~~ SUPERDEX® 75; Spherical composite of cross-linked agarose and dextran with a resolution range of 3000-70000 Daltons for globular proteins) fractions;

[0033] Fig. 8A is a chart of gel filtration (~~Superdex~~ SUPERDEX® 200; Spherical composite of cross-linked agarose and dextran with a resolution range of 10000-600000 Daltons for globular proteins). The bar and arrow pointed to it indicate the fractions, as shown in 8C, with MMP-9 inhibition activity. The two vertical arrows mark the gel exclusion fraction and the elution point for a size of 450,000 Dalton;

[0034] Fig. 8B is a chart showing the relationship between elution times on ~~Superdex~~ SUPERDEX® 200 (Spherical composite of cross-linked agarose and dextran with a resolution range of 10000-600000 Daltons for globular proteins) and molecular weights;

[0035] Fig. 8C is a chart showing MMP-9-inhibiting activities for the respective fractions obtained through ~~Superdex~~ SUPERDEX® 200 (Spherical composite of cross-linked agarose and dextran with a resolution range of 10000-600000 Daltons for globular proteins);

[0038] Fig. 11 is a chart showing MMP-9-inhibiting activity in the blood serum of a pancreatic cancer patient in Example 3. Arrow in the drawing identifies a low-molecular-weight V type collagen decomposition product produced by MMP-9 proteolysis; and

[0044] Thus, "a molecular weight of 500 kDa or more" refers to a molecular weight estimated by gel filtration chromatography, which is a molecular weight specifically

measured with ~~Superdex~~ SUPERDEX® 200 (Spherical composite of cross-linked agarose and dextran with a resolution range of 10000-600000 Daltons for globular proteins)in the range of from 500 kDa to the exclusion limit 1300 kDa.

[0083] The precipitate recovered by Step (6) consists essentially of the proteoglycan of the invention. The precipitate resulting from Step (6) may further be purified by gel filtration or the like depending on use. The method of purification may include isolating fractions with a molecular weight of 500 kDa or more by preparative gel filtration chromatography (for example, using ~~Superdex~~ SUPERDEX® 200 (Spherical composite of cross-linked agarose and dextran with a resolution range of 10000-600000 Daltons for globular proteins)). In the concentration step, membrane filtration with a cut-off value of 500 kDa may be performed in place of Step (8).

[0099] As shown in Fig. 4, the MMP-9-inhibiting activity was higher in fractions with acid isoelectric points and particularly higher in pH 2.0-3.0 fractions.

(8) Fractionation by ~~Superdex~~ SUPERDEX® 75 (Spherical composite of cross-linked agarose and dextran with a resolution range of 3000-70000 Daltons for globular proteins) gel filtration HPLC

[0100] Fractions with high MMP-9-inhibiting activity as a result of the isoelectric focusing of Section (7) were centrifuged (12,000 rpm, 30 minutes). The resulting supernatant was filtrated through a 0.45 μm filter, and then 200 μl of the filtrate was fractionated by gel filtration HPLC. The separation was performed using the conditions of an eluting solution of 50 mM Tris-HCl (pH 7.86) containing 200 mM NaCl and 10 mM CaCl2 and a column of ~~Superdex~~ SUPERDEX® 75 (Spherical composite of cross-linked agarose and dextran with a resolution range of 3000-70000 Daltons for globular proteins) (manufactured by Amersham Biosciences). The detection was performed at a flow rate of 0.5 ml/min and a wavelength of 230 nm (Fig. 5). Using FRACTION COLLECTOR (manufactured by Bio-Rad Laboratories, Inc.), a fraction was obtained every minute from 10 minutes immediately before a peak arose, and then each fraction was examined for MMP-9-inhibiting activity in the same manner as in Section (6) (Fig. 6).

[0103] Fig. 7 shows that the macromolecular fraction with the MMP-9-inhibiting activity was detected as a broad band before the pronase treatment but detected at the same position as chondroitin sulfate C (CSC) after the pronase treatment. Thus, it has been demonstrated that the acid, hydrophilic, macromolecular fraction with the MMP-9-inhibiting activity contains chondroitin sulfate C and is bonded to a protein.

I. (10) Fractionation by ~~Superdex~~ SUPERDEX® 200 (Spherical composite of cross-linked agarose and dextran with a resolution range of 10000-600000 Daltons for globular proteins) Gel Filtration HPLC

[0104] The shark cartilage extract obtained in Section (1) was dissolved in distilled water to form a 1 mg/ml solution. The solution was centrifuged (12,000 rpm, 30 minutes), and the resulting supernatant was filtrated through a 0.45 μm filter, and then 200 μl of the filtrate was fractionated by gel filtration HPLC. The separation was performed using the conditions of an eluting solution of 50 mM Tris-HCl (pH 7.86) containing 200 mM NaCl and 10 mM CaCl2 and a column of ~~Superdex~~ SUPERDEX® 200 (Spherical composite of cross-linked agarose and dextran with a resolution range of 10000-600000 Daltons for globular proteins) (manufactured by Amersham Biosciences). The detection was performed at a flow rate of 0.5 ml/min and a wavelength of 230 nm (Fig. 8). Using FRACTION COLLECTOR (manufactured by Bio-Rad Laboratories, Inc.), a fraction was obtained every minute, and then each fraction was examined for MMP-9-inhibiting activity in the same manner as in Section (6).